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NUTRITION REVIEWS

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SALT, FAT AND HYPERTENSION: THE JAPANESE EXPERIENCE

While pursuing studies linking salt intake to the development of essential hypertension (L. K. Dahl and R. A. Love, *A. M. A. Arch. Int. Med.* **94,** *525 (1954)*; *J. Am. Med. Assn.* **164,** *397 (1957)*; *New Engl. J. Med.* **258,** *1152, 1205 (1958)*), an opportunity was made available to study the adult population of a Japanese farm village in the vicinity of Hiroshima City during February through April 1958. At this time, it was discovered that the clinical picture of hypertension was significantly different from that commonly observed in the United States. On both clinical and laboratory grounds, these Japanese had strikingly little evidence of the usual cardiac complications found so commonly in western adult populations.

Among patients with chronic arterial hypertension who die of effects related to this disease, it has been generally accepted that the chief causes of death are due to the complications of atherosclerosis in the vessels of the heart, brain and kidneys. While it is recognized that there is no necessary relationship between these two diseases, in western society atherosclerosis, particularly of the coronary arteries, is often thought to be a well-nigh inevitable concomitant of chronic hypertension. There is little doubt that in our society hypertension accelerates the progress of established atherosclerosis and it seems likely that the onset of atherosclerosis is materially hastened by the presence of hypertension.

Relative to coronary artery disease for instance, an excellent long-term, continuing study in Framingham, Massachusetts, (T. R. Dawber, F. E. Moore and G. V. Mann, *Am. J. Pub. Health* **47,** *4 (1957)*) indicated that over a four-year period males aged 45 to 62 with antecedent hypertension developed about three times more clinical coronary artery disease than did males of similar age without hypertension. However, the affected individuals appear to have been on what might be called an atherogenic regimen, *i.e.*, a type of life in which atherosclerosis could be expected to develop in the absence of hypertension.

What is the situation when individuals with hypertension are on a nonatherogenic regimen? Most of the available experimental evidence with animals indicates that on normal diets, atherosclerosis does not develop following prolonged hypertension induced in the rat, dog, rabbit, sheep, goat and probably the chick. By contrast, when changes are effected in lipid metabolism through modifications of diet in the rabbit and chick (in the case of the dog, with the production of hypothyroidism as well), atherosclerosis develops which is clearly intensified and accelerated when the factor of hypertension is added (L. N. Katz and J. Stamler, *Experimental Atherosclerosis, p. 224. Charles C. Thomas, 1953*).

The fact that animals with chronic experimental hypertension ordinarily do not develop atherosclerosis suggests the possibility that under certain conditions, human hypertension may be dissociated from this companion disease.

I. Snapper reported that among the Chinese patients at Peking Union Medical College, atherosclerosis was uncommon and when hypertension was present, it was not associated with coronary thrombosis, although heart failure and cerebral hemorrhage were still seen (*Chinese Lessons to Western Medicine, p. 171. Interscience, 1941*). It is not clear from his brief discussion just how common either hypertension or the named complications were.

A human situation analogous to that of animals with experimental hypertension may be present in Japan where hypertension is a common and serious disease but coronary artery disease is rare, at least among the non-

professional, laboring majority of the population. In Japan, deaths from hypertension follow primarily cerebrovascular accidents as opposed to western society where deaths are largely due to cardiac complications. For instance, in B. J. Clawson's well-known large autopsy series in the United States (*Hypertension: a Symposium, p. 239. Univ. of Minnesota, 1951*), among those who were classified as dying of causes related to hypertension, 80 per cent died primarily of heart disease and only 14 per cent of cerebral hemorrhage. By contrast, in Japan at least half of the hypertensive deaths in autopsied series are due to cerebrovascular accidents (H. Ueda, *Japan Med. J.* **1740,** *27 (1957)*).

Indeed, these aftermaths of hypertension have been the leading cause of death in Japan since 1951, and one of the leading causes since 1900 (S. Watanabe, *A Brief Report on Public Health Administration in Japan, p. 13. Japanese Ministry of Health and Welfare, 1956*). In 1955, Japanese males of the age group 35 to 80 had two to four times the mortality rate from this cause than American males of similar age, while the mortality from arteriosclerotic heart disease in male Japanese in this age group was from a third to a sixth as common as in their American counterparts (*Annual Epidemiological and Vital Statistics, 1955, pp. 406–411. World Health Organization, 1958*).

It is probable that differences in diagnostic standards account for some of the discrepancies in the incidence of these diseases in the two races, but it is difficult to reconcile the variations on this basis alone. For instance, in one autopsy series of about 10,000 Japanese the incidence of severe coronary atherosclerosis was approximately one tenth that of comparable individuals in the United States (N. Kimura, *Cardiovascular Epidemiology, p. 22. Hoeber-Harper, 1956*).

Furthermore, unpublished observations by American physicians of the Atomic Bomb Casulty Commission in Hiroshima are in accord with the belief that in contrast to hypertension, coronary heart disease is relatively uncommon in the Japanese. Among the 5000 adults studied during the years 1950–55, a diagnosis of arteriosclerotic heart disease was made in 45 patients, infarction of myocardium secondary to arteriosclerotic coronary thrombosis in three, and infarction due to unknown cause in an additional eight patients. In this same series, a diagnosis of hypertensive cardiovascular disease was made in 227 patients, hypertensive vascular disease in 293, and essential vascular hypertension in 430 (Crowley, F. B., Jr., *personal communication. ABCC, Hiroshima*).

Interestingly, while the incidence of cerebrovascular accidents is generally high throughout Japan, there seem to be areas in which it is much higher than others. In general, the highest rates are in the northern areas of the main island of Honshu with progressively lower rates going southward (E. Takahaski *et al., Human Biol.* **29,** *139 (1957)*). This disparity in distribution is not explained by differences in climatic conditions, since the most northerly island of Hokkaido has less than half the rate of the areas noted above in Honshu. The areas in which cerebrovascular accidents are most common are also those in which hypertension is most common.

The primary cause of the cerebrovascular complications, which appear to be so common in the hypertensive Japanese, is less clear. If the lesions are primarily atherosclerotic in origin, this might suggest that the pathogenesis of coronary and cerebral atherosclerosis may be somewhat different.

If the lesions are not atherosclerotic, what are they? No clear-cut answer is available at present, but recent discussions (R. D. Adams, *Cerebral Vascular Diseases, Second Edition, p. 23. Grune & Stratton, 1958*) of hypertensive cerebrovascular disease have reemphasized the evidence for nonatherosclerotic processes such as aneurysm formation from weakened vessels. Certainly the relative mildness of atherosclerosis in the other areas of the body argues for the non-

atherosclerotic origin of some Japanese cerebrovascular accidents. It would also aid in explaining the well-known fact that, while American men have much more mortality from atherosclerotic heart disease than American women, the mortality from cerebrovascular lesions is very nearly equal in the two sexes (*Annual Epidemiological and Vital Statistics, 1955, p. 407. WHO, 1958*).

It is doubtful whether genetic factors are responsible for the striking difference in the incidence of coronary artery disease in Japanese and Westerners, although assessment of this factor is incomplete. It has been reported that the mortality rate from arteriosclerotic heart disease was higher among male Japanese living in Hawaii than among comparable men in Japan. However, Hawaiian Japanese had less disease than Japanese in continental United States, and these, in turn, had less than white Americans of similar age (T. Gordon, *U. S. Pub. Health Reports* **72,** *543 (1957)*). Since in this study most of the Japanese males living in Hawaii and the United States in the susceptible age group, 45 to 64, were immigrants from Japan, it is probable that such individuals remained influenced by cultural (*e.g.* dietary) patterns already established. Moreover, it is well-known that Hawaiian Japanese have retained more of their native customs than have those who migrated farther west.

A study of males of Japanese ancestry who have been exposed to a strictly western environment from birth will be a more critical test, and one might predict that arteriosclerotic heart disease will prove to be the scourge it now is among most other adult American males. Correspondence with cardiologists in Los Angeles and Honolulu suggests that this may be true at present.

There have been numerous western influences in Japan, especially since World War II, and this has included some change in diet, particularly in the large cities among the professional classes. However, the great majority of Japanese still consume the classical Japanese diet, and the current interest in dietary factors which may play a role in the pathogenesis of atherosclerosis and hypertension warrants a passing description of this diet. From the American viewpoint, it might be most simply described as a primarily vegetarian, low fat (largely unsaturated), average protein, high carbohydrate, high salt diet (*Malnutrition and Nutrition Activities in Japan, p. 3. FAO/WHO Nutrition Committee. Japanese Ministry of Health and Welfare. 1956*). The daily fat intake of about 20 to 30 g. is derived almost exclusively from vegetables and fish and accounts for about 10 per cent of the caloric intake. Protein intakes are about 70 g. per day, of which only a quarter comes from animal sources, mostly fish and shellfish. Calories are derived mainly from cereals, of which rice is the principal source.

Salt intake is generally high and appears to decrease from north to south. In two separate studies (T. Fukuda, *J. Med. Soc. of Chiba* **29,** *490 (1954)*; N. Sasaki, *Japanese J. Hygiene* **13,** *11 (1958)*), farmers in northern Honshu were found to eat about 27 g. per day, while those in middle and southern Japan averaged about 17 g. per day. (American males eat about 10 g. per day.) It is of some interest that the areas in which salt consumption is greatest appear to coincide with those areas where the prevalence of hypertension and cerebrovascular accidents is greatest (E. Takahaski *et al., loc. cit.*).

If high fat and high salt intakes are important etiologic factors in the development of atherosclerosis and hypertension, respectively, it could be predicted *a priori* that the Japanese would be afflicted primarily with hypertension rather than atherosclerosis. This seems to be true.

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NUTRITION FOR MAN IN SPACE

The weight of nutrients needed to supply man in space becomes a tremendous problem when flights are extended for more than a few weeks or months.

Food and drink has always been an important item for travelers, particularly in unfamiliar environments. At first the traveler's main interest is in obtaining sufficient nutrition to sustain life. After this a varied diet to eliminate monotony and supply taste preferences becomes important, and finally, if there is a plethora of nutrients, exotic foods are sought. The supplying of wholesome and appetizing foods for persons traveling on land, sea and in conventional aircraft has been solved in a relatively easy manner. However, with the advent of what the military calls high-performance aircraft, new problems of providing the travelers with desirable feeding programs are created.

Recently B. Finkelstein and A. Taylor (*Military Med.* **124,** *725 (1959)*) have reported some of the problems involving nutrition for people in space vehicles. While their immediate goal is the feeding of military personnel who will be manning the aircraft of the very near future in flights of short duration, the experience gained from their studies will be used as a basis for designing feeding programs for future long-term space travel.

The type of feeding program needed for space travel will depend entirely upon the length of the flight. These authors divided the duration of flight into three categories: those of short duration (two to three days), those lasting more than three days and up to an indefinite number of months, and finally, voyages of long duration lasting for years or even generations. One of the problems of feeding under such defined flight schedules concerns the weight not only of the food itself, but of the containers and the food handling equipment and utensils.

Oxygen, not normally considered a nutritional problem, must also be supplied to the travelers. The weight of oxygen used by man is approximately one and a half pounds per day (depending on activity), or approximately 550 pounds per year. Since man needs approximately 2.2 liters of water per day, one would have to carry almost a ton of water to supply one man for a year. Moreover, it has been calculated that an individual at home consumes approximately seven pounds of food per day, which includes the weight of the packaging, inedible portions and food lost in preparation.

Finkelstein and Taylor report how some of these problems have been solved, at least in part. For flights of only two or three days, bottled oxygen, stored water, ready-to-eat types of food (box-lunch style) prove adequate. For longer journeys, the weight problem may still be solved by present technology if the length of flight is not too long. This requires devices which break down carbon dioxide to oxygen so that it can be re-used. Water can be recovered from both the air and excreta and purified. Dehydrated foods packaged in very light containers from which the food can be eaten and equipment limited to a means of heating the package will reduce the weight of the food load drastically.

However, for flights of long duration a ration of dehydrated "quick serve" foods supplying about 2500 calories per day with the usual ratio of fat, carbohydrate and protein and adequate amounts of minerals and vitamins would weigh about 3.5 pounds. To supply one man with this type of food for one year would add about 1300 pounds of weight. Thus, for trips of longer than two to six months it would not be economical to carry foods.

Methods must be developed for regenerating carbon and nitrogen compounds and reconverting the metabolic wastes into food. A process of growing algae can be used for such a cycle, which will also produce oxygen.

In order to operate such a scheme in a space vehicle, however, the natural process must be sped up greatly and confined to a small space. While the basic research in this field already has been done, considerable increase in technology or applied research must be undertaken before such a process becomes feasible.

As a prelude to this long-term space travel, Finkelstein and Taylor have studied the problem of feeding personnel who would be manning high-performance aircraft. They studied two different groups of five men who were maintained in the confined limits of a crew compartment for five days, and also 35 individuals who were maintained in a dark sound-proof chamber from six to 168 hours. These studies simulated some of the conditions of space travel to which crews would be subjected. A study was also carried out on a subject during a high-altitude balloon flight of about 100,000 feet. In all these investigations it was possible to provide adequate nutrition under rather stringent environmental conditions.

In contrast to the normal feeding of military personnel, however, consideration must be taken of the psychological effect of food. It has been found that under stressful situations food may serve as a means of relieving stress.

Under the conditions of true space travel man will be subjected to periods of weightlessness, a condition which, according to these investigators, cannot be reproduced experimentally for more than about 45 seconds. Thus at present one cannot study the effects of lack of gravity on the mechanics of eating, ease of digestion, absorption or even gross nutritional requirements. However, it is possible to surmise some of the difficulties and plan accordingly.

During early ventures in space, man will probably wear the full pressure suit, which includes helmet and gloves. Thus in order to feed him, it will be necessary to employ fluid diets contained in squeeze-type bottles which can be connected to a tube leading to the mouth. The particle size of the formula must be small to provide free flow and also to promote better digestion. When normal gravitational effects are removed for short periods there have been reports of disorientation, nausea and motion sickness. With the use of liquid foods under gravity-free conditions, measures must be taken to prevent the aspiration of the diet into the lungs. Also, the diet must be low in residue since pressure suits make no provisions for defecation. The group has already reported two different fluid menus which supply 2300 calories per day with a protein intake of 100 g. Test subjects on such a regimen carried out their normal duties and had insignificant weight changes during the period studied.

These investigators conclude that, while the problem of feeding personnel in high-performance aircraft and in space travel has not yet been solved, greater variety of concentrated foods, further advances in cold sterilization of foods, improved methods of packaging and the development of lightweight food service equipment will provide some solutions.

SYNDROMES OF MAGNESIUM DEPLETION AND RETENTION IN MAN, PART II

A syndrome of magnesium deficiency can probably be defined in certain patients with alcoholism or electrolyte imbalance. This consists of sudden psychoses, neuromuscular hyperirritability and electrocardiographic changes.

As pointed out in part one of this review, recognition of clinical magnesium deficiency has been hampered by the poor correlation between serum and intracellular concentrations of this ion. Investigators and clinicians can at best make inferences until some more

direct measure of intracellular levels is worked out.

Precisely this has been attempted by W. O. Smith and J. F. Hammarsten in an article entitled "Intracellular Magnesium in Delirium Tremens and Uremia" (*Am. J. Med. Sci.* **237,** *413 (1959)*), in which they have shown very nicely changes in cell magnesium content in these two syndromes. The authors refer to a number of papers implicating an increase or decrease of magnesium in certain clinical situations, principally delirium tremens and uremia. They point out that the poor correlation between serum magnesium levels and these syndromes is analogous to the inconsistent results reported in the earlier papers on potassium. Smith and Hammarsten selected red blood cells as the most easily obtainable cells for their determinations, recognizing, however, that erythrocytes might not be typical of other cells of the body.

In 13 healthy adults the mean erythrocyte magnesium concentration was 5.29 ± 0.34 mEq per liter; the corresponding plasma level was 1.80 ± 0.13.

Twelve patients with delirium tremens were studied. All were disoriented and confused; 11 had a "pronounced" tremor and seven were suffering hallucinations. The mean red cell magnesium concentration was 3.9 ± 0.75 mEq per liter, and all values were below the "normal" range defined on the authors' charts. The mean plasma concentration was 1.50 ± 0.28 mEq per liter, a significant decrease ($p = < 0.001$), although five or six of the values were within the "normal" range of 1.54 to 2.06. In the nine patients treated with intramuscular magnesium sulfate there was "marked" clinical improvement within 12 to 24 hours. Seventy-two hours after this treatment, the mean red cell magnesium concentration had risen to 5.19 ± 1.47 mEq per liter and the mean plasma level to 2.20 ± 0.40. The individual values are not given in the paper.

Determinations were made on 14 patients who had uremia and central nervous system depression. The mean erythrocyte magnesium concentration was 8.84 ± 1.71 mEq per liter, and all 13 values charted were above the upper limit of normal (6.00). The mean plasma level was 3.17 ± 1.30 mEq per liter; ten of the 14 values were above the upper limit of "normal" on the chart and the remaining four were within the normal range.

Electrocardiograms were made on three patients with delirium tremens and nine of the uremic patients. Changes possibly attributable to abnormal intracellular magnesium concentrations were found in two thirds of these patients.

While the authors are cautious about generalizing, they conclude that at least one cell, the erythrocyte, is depleted of magnesium in delirium tremens and that "the close correlation between the hyperexcitability of the central nervous system with the lowered erythrocyte magnesium levels suggests that the brain cells may also be deficient in magnesium."

What the correlation between magnesium levels in erythrocytes and other cells may be is not discussed. The concentration in muscle cells is several times that in erythrocytes, but one does not know what obtains in various other specific tissue cells, nor whether these values change independently or in parallel fashion.

Smith and Hammarsten point out that magnesium therapy of delirium tremens requires 12 to 24 hours before clinical improvement occurs and they speculate that the delay may be due to slow cellular repletion. They point out that serum or plasma magnesium concentrations are more reliable evidence of a total body excess than of a deficiency of magnesium, as would be expected from the predominantly intracellular position of this cation.

The authors' results, although derived from a small series of cases, seem quite significant. First, needed data have been provided to those interested in electrolyte physiology. Second, the results establish

what previously had only been surmised from a confusing assortment of clinical observations and plasma levels. Lastly, they are quite consistent with what we know of the physiologic actions of magnesium. The parallel with the development of our knowledge of syndromes of potassium excess and deficiency may well be a close one.

Smith and Hammarsten are careful not to claim that the changes they have found are the cause of delirium tremens or the uremic syndrome, but it seems reasonable to believe that they are important factors. Further research will establish whether this is so.

Further work on magnesium depletion in man is reported by R. E. Randall, Jr., E. C. Rossmeisl and K. H. Bleifer (*Ann. Int. Med.* **50,** *257 (1959)*). They report observations on 12 patients with sudden psychiatric and neuromuscular symptoms and relate the symptoms to depletion of total body magnesium; "in ten instances the serum magnesium concentrations were notably depressed." Several patients showed evidence of depletion of body magnesium, and calcium metabolism was regularly altered. The authors cite normal serum magnesium values as 1.7 to 2.7 mEq per liter (mean 2.2). Postmortem examinations were performed in six cases and surgical biopsies in two others.

Case 2 was a 68-year-old man with carcinomatosis, severe malabsorption and cachexia. An abdominoperineal resection was performed for carcinoma of the rectum in 1950. Heavy irradiation for metastases in 1955 was followed by diarrhea and, in 1957, cachexia. Laparotomy and biopsy showed carcinoma and "extensive post-irradiation changes"; the entire small bowel was scarred and adherent. After a period at home with continuing diarrhea and weight loss, the patient was readmitted because of weakness and "episodes of bizarre behavior." On the following day he "suddenly became irrational, disoriented, confused, and progressively noisy, combative and wildly restless," with hallucinations. Intravenous fluids were given, with vitamins, dextrose, saline and potassium. After six days of severe psychosis "a severe neuromuscular disorder developed, characterized by muscular rigidity and cogwheeling, coarse tremor, opisthotonos, extensive fine muscle fasciculations and twitching." Very low serum levels of calcium and magnesium were discovered. Intravenous calcium gluconate caused no improvement.

Nine days after onset of the psychosis, intravenous magnesium sulfate was added to the regimen (150 mEq plus that in the diet). Eighteen hours after the first dose of magnesium sulfate "the patient was remarkably rational, oriented, and completely free of the previous neuromuscular disorder. By the third day of therapy he was up and about, completely transformed from his 'terminal' state into a delightful elderly man." Over the next five weeks, with diarrhea and no supplement of magnesium, the serum calcium and magnesium levels fell progressively again to very low levels but with no psychiatric or neuromuscular symptoms. Changes in the electroencephalogram and electrocardiogram improved slowly after treatment, but could not be correlated with electrolyte levels or clinical symptoms.

Case 4 was a 63-year-old alcoholic man with cirrhosis. Part of the colon was removed (because of multiple polyps); there followed much gastric and fecal fluid loss, confusion on the second postoperative day and severe psychosis and twitching by the fourth. Treatment with magnesium was followed by improvement within 15 hours. Abnormalities in the electromyogram, electroencephalogram and electrocardiogram disappeared and much of the magnesium was retained.

Case 5 was a 38-year-old diabetic male with mild chronic pyelonephritis, admitted to the hospital because of a stroke and aphasia. There had been no gastrointestinal or dietary abnormality. The patient developed severe epilepsy ("status epilepticus") on the third day, lasting for three

weeks and uncontrolled by large doses of anticonvulsant drugs. When extreme serum electrolyte changes, including very low magnesium values, were found, large amounts of several electrolytes, including calcium, potassium and magnesium, were infused. "During the first hour of electrolyte 'replacement' the status epilepticus stopped and only focal seizures occurred." Although magnesium was retained during the first 40 hours, there followed three days of large renal losses. However, there was a large retention of magnesium several times during the second and third months. The patient improved rapidly and within three weeks could walk, with residual right hemiparesis and speech defect. Electrocardiographic and electroencephalographic findings improved progressively.

Three months after admission to the hospital a myocardial infarct supervened, leading to death 11 days later. Postmortem examination showed the infarct of the brain and heart and acute and chronic pyelonephritis; the intestine was normal. Although the authors are unable to explain the marked changes in calcium metabolism, they presume that the potassium depletion was a renal loss superimposed on a deficient intake. In some way, they infer, this may have brought about simultaneous renal loss of magnesium.

Randall and his co-workers, summarizing their cases, found that all patients were "severely malnourished" for such reasons as anorexia, inability to eat, alcoholism, diarrhea and vomiting. Hypokalemia was a feature of three cases and two had hyponatremia. Eleven of 12 patients were being fed parenterally when their symptoms developed or became worse. Seven patients had sudden and severe psychoses ("delirium, hallucinations, delusions and wild, combative behavior"); four others had "mild confusion and disorientation." Withdrawal from alcohol immediately before hospital admission may have occurred in several of the alcoholic patients. Muscle twitchings occurred in all but one patient; rhythmic tremors occurred in six, seizures in two, and only one (case 5) had physical signs of neuromuscular irritability. Ten patients had low serum magnesium levels; two had normal levels with balance data indicating magnesium depletion. Hypocalcemia was found in all 12 patients.

Psychotic and neuromuscular symptoms stopped in six cases within six to 18 hours after treatment was started; improvement was "dramatic" in three of these (including cases 2 and 4, summarized above). In several cases the symptoms and serum magnesium levels could not be correlated. All six patients tested had "diffusely abnormal" electroencephalographic tracings. Many showed depressed ST segments and low T wave voltage in the electrocardiograms. Six autopsies and two biopsies yielded no specific lesion attributable to magnesium deficiency.

Magnesium tetany (in a child) has been reviewed in this journal (*Nutrition Reviews* **2,** *189 (1944)*). Recently, D. D. Ulmer, W. E. C. Wacker and B. L. Vallee have reported four patients (*J. Clin. Invest.* **38,** *1049 (1959)* (abstract)). "True magnesium tetany" occurred in each case "as a complication of either severe infection or surgery in malnourished patients, treated with magnesium-free parenteral fluids for prolonged periods. In each, the serum magnesium concentration was low and that of calcium was normal. Parenteral magnesium resulted in prompt and dramatic amelioration of all the manifestations of the *magnesium tetany* syndrome."

The authors found the syndrome "virtually identical" to that observed in animals: "semicoma, severe neuromuscular hyperirritability including Chvostek's sign and carpo-pedal spasm, athetoid movements, marked susceptibility to auditory, mechanical and visual stimuli, a decreased magnesium and a normal serum calcium concentration." The syndrome "can only be distinguished from hypocalcemic tetany by chemical means." The importance of the observations of Ulmer's group lies in the

apparently more discrete clinical identification of the background for the magnesium deficiency. Their full report should prove illuminating.

The various authors cited in this review have reported an impressive amount of clinical research. The studies are perforce incomplete and leave many questions unanswered.

Simplified methods of magnesium determination (flame photometer, spectrography and chelation) are being developed and should before long be widely used to answer these questions. Should flame photometry, for example, prove to be a really simple and reliable method of measuring magnesium in biological fluids, one may expect many new observations among patients with electrolyte disturbances, primary aldosteronism, acute renal failure, hyperparathyroidism and others, as well as in the whole gamut of conditions associated with altered neuromuscular irritability.

The difficulties caused by the intracellular location, large body stores and renal conservation of magnesium will have to be overcome. Randall and co-workers point out that estimation of total body magnesium (*e.g.*, by the radioisotope dilution method) would be a far better measure of depletion than are balance data. The lack of correlation between serum magnesium concentration, total body stores and intracellular levels has been emphasized by all authors. No doubt erythrocyte content as measured by Smith and Hammarsten is a better indicator, but we do not yet know how satisfactory it will prove.

The known pharmacologic relations between magnesium and potassium and calcium metabolism have never been well explained; perhaps they will be soon. There is evidence that calcium or potassium administration aggravates magnesium deficiency in animals. Randall's group points out that decreases in serum magnesium levels have been observed in man after calcium administration and after glucose. Perhaps the latter phenomenon is a result of increasing intracellular glycogen, entraining intracellular ion with it. The authors point out that some of their patients had been receiving glucose, potassium or calcium in their parenteral fluids. Much remains to be elucidated before the relation of magnesium to calcium metabolism is worked out, and our speculations about bone deposition, intracellular content, phosphate, vitamin D and parathyroids are very imperfect at present.

The papers reviewed have helped considerably to define the syndrome of magnesium deficiency in man, which should soon qualify for inclusion in our textbooks. If patients with such a syndrome can be distinguished, our knowledge can be greatly advanced by the mere selection of subjects appropriate for study.

It seems fair to conclude that onset of psychosis or semicoma plus signs of tetany, especially in circumstances with starvation and fluid and electrolyte loss, should suggest the possibility of magnesium deficiency. In short, if the stage is set, onset of a delirium tremens-like syndrome justifies a chemical and metabolic search for magnesium deficiency as a prelude to specific replacement therapy.

IRON FORTIFICATION OF MILK

The fortification of a prepared milk formula with iron (12 mg. per reconstituted quart) has been shown to maintain hemoglobin and serum iron concentrations at normal levels in both full-term and premature infants.

Many recent studies have emphasized the prevalence of iron deficiency in infants between six and 18 months of age in the United States (*Nutrition Reviews* **17,** *11*

(*1959*)). Since iron deficiency in infancy is a preventable disease, this large public health problem can be solved by the addition of adequate amounts of iron to the infant dietary. Studies by the Child Research Council in Denver (*Ibid.* **16,** *67* (*1958*)) have demonstrated that infants ingesting large amounts of infant cereal fortified with iron have intakes generally considered to be adequate for the prevention of iron deficiency anemia.

W. L. Niccum, R. L. Jackson and G. Stearns (*Am. J. Dis. Child.* **86,** *553* (*1953*)) demonstrated that the addition of inorganic iron to milk in amounts of 5 to 10 mg. daily would also prevent iron deficiency anemia. However, studies on the absorption of iron from milk conflict with studies in which iron is administered separately.

The following study demonstrates quite clearly that iron added to milk is absorbed in amounts adequate to prevent the development of iron deficiency anemia and to maintain serum iron concentrations that must be considered normal for infants.

A. Marsh, H. Long and E. Stierwalt (*Pediatrics* **24,** *404* (*1959*)) studied the performance of infants during their first nine months of life on three feeding regimes. Group one was fed a prepared milk formula containing 12 mg. of iron per quart. The second group was fed the same prepared formula without added iron. A third group was fed a formula made from evaporated milk, water and sucrose at the same caloric concentration and containing no added iron. Except for small amounts of cereal of extremely low iron content, no solid foods were received by these infants during the nine months of the study. Seventy-four full-term and 42 premature infants were observed for the full nine-month period.

The hemoglobin values for the full-term infants were consistently higher in those fed the iron-containing formula after the age of three and one half months, averaging 12.69 g. per cent for the iron-supplemented group and 10.46 and 9.67 g. per cent for the unsupplemented groups. These differences were highly significant on statistical analysis. Among the premature infants, the iron-supplemented group had hemoglobin concentrations practically identical with those of the full-term infants but the hemoglobin concentrations of the unsupplemented groups were even lower, averaging 9.4 and 8.5 g. per cent respectively for groups two and three.

Serum ion concentrations were also measured. In the full-term infants, statistically significant differences between the iron-supplemented group and the two control groups appeared after the age of five months, and as early as the age of three months in the premature infants. Statistically significant differences in the hematocrit occurred only at the age of nine months and were not as pronounced as the differences in hemoglobin or serum iron concentrations.

The iron-fortified milk preparation used was well tolerated by all the infants in the study. No objective evidence of a difference in incidence of infection or other criteria of performance was found. Mean serum iron concentrations in the supplemented group averaged about 60 micrograms per cent for both full-term and premature infants. In the unsupplemented group, serum iron concentrations fell to approximately 35 micrograms per cent in the full-term infants and to as low as 20 micrograms per cent in the premature infants. These low serum iron concentrations were not always accompanied by the development of anemia (defined for the purposes of this study as a hemoglobin concentration less than 8.0 g. per cent).

These studies were carried out under somewhat artificial conditions since supplementation of the milk diet for the first nine months of life was not allowed in the study group. The demonstration that iron added to a prepared formula at a concentration of 12 mg. per reconstituted quart will maintain normal hemoglobin concentrations in infants has confirmed previous studies,

supplementing them with data concerning the concentration of serum iron.

Although there is no doubt that iron deficiency can be prevented by the addition of iron to the milk formula of both premature and full-term infants, other foods can also be supplemented. Certainly a great deal more experience concerning supplementation of infant dietaries needs to be accumulated before the wholesale fortification of the milk supply available to small infants can be seriously considered.

MINERAL ELEMENTS OF FRESH VEGETABLES

The average amounts of ten mineral elements in fresh vegetables vary widely between vegetables from different geographic areas and even from shipment to shipment from the same farm.

Ever since the recognition of deficiency diseases, there has been concern regarding the composition of foods from different areas. The effects of native fertility, soil amendments and other environmental factors upon the composition of vegetables and fruits have been scrutinized as possible indicators of food quality.

With Washington, D. C. as a marketing center, an extensive study has been made of the mineral composition of nine vegetables from producing centers located in California, Arizona, Texas, New Mexico, Maryland, New Jersey, New York, Michigan, Delaware, Florida, North Carolina and Pennsylvania (H. Hopkins and J. Eisen, *J. Agr. Food Chem.* **7**, *633 (1959)*). Three or more lots of each vegetable were collected equally from major producing areas representing 90 per cent of the wholesale market during 1956 and 1957, with the exception of sweet corn and tomatoes. The seasonal delivery pattern limited collection of samples to five different vegetables at any one time, and efforts were made to secure samples shipped by different producers in a given area when samples were secured in successive weeks. Information on production and handling was secured from the label and shipping tags and from the shippers and growers.

A crate, bushel or carton of each vegetable was secured and reduced to the edible portion for analysis. The vegetables analyzed included asparagus, snap beans, cabbage, carrots, pascal celery, sweet corn, iceberg lettuce, onions and tomatoes. These make up the bulk of vegetables, other than potatoes, used in United States households (*U. S. Dept. Agr., A. M. S. Household Food Consumption Survey Report No. 1 (1955)*). The elements determined included boron, phosphorus, magnesium, manganese, iron, aluminum, calcium, copper, sodium and potassium.

In presenting their report, Hopkins and Eisen emphasize that there may be large variations in the amount of an element found in different vegetables, or in the same vegetable from different areas, or in the same vegetable from the same area at different seasons, or indeed in the same vegetable from the same farm and the same season but from different shipments. Earlier studies have also noted the differences which occur for certain elements between lots, and G. T. Sims and G. M. Volk pointed out that these differences limited the significance of regional differences (*Fla. Agr. Expt. Station Bull.* **438** *(1947)*).

The rapid changes which have occurred in cultural practices within the last decade give special pertinence to the values presented by Hopkins and Eisen. The wide diversity of production areas, the selection of vegetables and the ten mineral elements for which values are reported, lend additional interest to their report. Also, most of the values for a specific element in a given vegetable had a

coefficient of variation of less than 10 per cent, suggesting a good sampling procedure.

The levels of sodium found in different areas were quite striking. Carrots and celery were particularly noteworthy, in that they had much higher values on the average than the other seven vegetables. Carrots averaged 53.0 mg. per 100 g. with a low value of 1 mg. and a high of 132 mg. per 100 g. Celery ranged from 27 to 133 mg. per 100 g. Unfortunately for those who might like to select these two vegetables for sodium content, some of both high and low values came from the same areas. However, the sodium content of the other vegetables was of an entirely different magnitude. For example, the average value of sweet corn was 0.19, tomatoes 2.1, asparagus 1.8 and iceberg lettuce 6.3 mg. per 100 g. of edible portion.

There were some large differences in area mean values for some of the elements in certain vegetables. The mean copper content of carrots from northwest New Mexico (0.05) may be compared with that of South California (0.18); the sodium of central California iceberg lettuce (13.0) with that of central New York (0.9); the manganese in tomatoes from central Florida (0.09) with that in tomatoes of Eastern Pennsylvania (0.21), all in terms of mg. per 100 g. of edible portion. However, the wide variations which may occur even with successive deliveries from the same farm emphasize the hazard of attributing any special significance to regional differences.

Hopkins and Eisen gave special attention to the variations in phosphorus and manganese content of fresh carrots as received in Washington, D. C. during the period April–October 1957. The phosphorus values ranged only slightly about the weighted mean of 34 mg. per 100 g. fresh weight during this period, and this was characteristic of most of the other elements. The manganese values, however, varied quite widely around the weighted mean of 0.29 mg. per 100 g., indicating a highly variable seasonal pattern for this element. A similar exceptional pattern is revealed for sodium in lettuce.

The data presented by Hopkins and Eisen partially fill a gap that has long been evident. The selection of material on an edible portion basis and from quite diverse production areas further enhances the values obtained. It is also clear that the variations in mineral element content, while not great for some elements, are tremendous for others.

Results such as these emphasize how little quantitative data are available concerning the mineral element composition of our foodstuffs, and how much less is known of the factors influencing the amounts of elements present in vegetables which come to market.

ANTAGONISM BETWEEN ESTRONE AND PARATHYROID EXTRACT IN CALCIUM METABOLISM OF BONE

A technique employing radioactive calcium permits detailed studies in animals of the rate of deposition in bone and of the size of the exchangeable pool. Parathormone and estrogen have opposite effects on calcium metabolism.

Many methods have been employed to estimate the status of skeletal metabolism of calcium. In far advanced rickets or osteomalacia, the clinical appearance is sufficient evidence to establish the nature of the defect. But in mild forms of skeletal disorders, sensitive tests must be employed. Unfortunately no single test or combination of these satisfies the requirement. A helpful method is provided by comparison of x-ray films of skeletal structures with films of a standard series of ivory (or metal) wedges of increasing thickness (*Nutrition Reviews* **10**, *119 (1952)*). However, even these tech-

niques fail to correlate perfectly with studies of calcium balance. Moreover, even the balance technique can be subject to misinterpretation, since the size of the exchangeable calcium pool will vary from one individual to another, and the rate of calcium absorption from the gut may vary from day to day.

Clearly, a more precise method was needed. Some of this gap was filled by a technique devised by G. C. H. Bauer, A. Carlsson and B. Lindquist (*Kungl. Fysiograph. Sälsk. Lund. Förh.* **25,** *1 (1955)*). This method, modified slightly by R. E. Ranney (*Endocrinology* **64,** *783 (1959)*), consisted of feeding a standard diet to adult male mice, administering a drug to be tested for an appropriate period of time, and injecting the animals with radioactive calcium (Ca^{45} lactate) at intervals of 144, 96, 48, 24 and four hours before sacrifice. Samples of serum and of bone (left tibia) were taken for radioactive measurement. The technique allowed estimates to be made of both the exchangeable pool of calcium and of calcium which had been deposited recently in bone (calcium accretion).

While endocrine control of calcium metabolism is certain, the details are in controversy. Studies of the pharmacologic effects of parathyroid hormone have shown that it increases the concentration of calcium in the blood and decreases that of phosphorus, while at the same time both substances are excreted more rapidly in the urine (F. Albright and E. C. Reifenstein, Jr., *Parathyroid Glands and Metabolic Bone Disease. Williams and Wilkins Co., Baltimore (1948)*). These effects may be accounted for by an increased rate of renal excretion of phosphorus. This leads to increased resorption of calcium phosphate from bone, and the consequent rise in levels of calcium in the blood leads to more rapid urinary loss of calcium. Unless this loss is compensated by greater dietary intake, the result will be decalcification of bone.

In opposition to this theory, W. F. Neuman and M. W. Neuman (*Am. J. Med.* **22,** *123 (1957)*; *Nutrition Reviews* **15,** *188 (1957)*) proposed a different explanation. Their view was that bony matrix is constantly being calcified as a result of exposure to fluids which are supersaturated with calcium salts. Balance is maintained by citric acid, formed by osteocytes in the bone, which chelates calcium and returns a portion of it to the blood. The action of parathormone would be to regulate the rate of formation of citric acid by these cells of the bone.

The role of estrogens in calcium metabolism has been equally controversial. While few deny that estrogens exert some effect, the mechanism has remained in dispute. Postmenopausal osteoporosis develops in some women deprived of their ovaries either by surgery or by senescence. Administration of estrogens (usually with androgens) may effect a cure. However demineralization of bone may occur during pregnancy, although this is presumably caused by inadequate diet.

In 1957, G. Manunta, J. Saroff and C. W. Turner (*Proc. Soc. Exp. Biol. Med.* **94,** *785 (1957)*; *Nutrition Reviews* **15,** *343 (1957)*) reported studies performed with the Ca^{45} technique of Bauer. Administration of estradiol resulted in more radioactive calcium in the serum but not in bone, while parathyroidectomy reduced Ca^{45} in bone but not in serum. When estradiol was given to parathyroidectomized animals it increased the Ca^{45} content of serum and prevented the decrease in bone. Parathyroid extract alone decreased the Ca^{45} content of bone, but estradiol and parathyroid extract together increased the Ca^{45} content of bone. These findings were interpreted as evidence that parathormone and estrogens have an opposite effect on bone metabolism and that estrogens might alter the transport of calcium.

Electrophoretic studies of serum from these animals showed that Ca^{45} migrated with albumin and the alpha globulins in control animals, but with beta and gamma globulins in animals that had received estrogens. Unfortunately some doubt was cast upon the validity of this latter finding be-

cause of the tendency of calcium to dissociate from its carrier proteins during electrophoresis.

In an attempt to clarify the effects of estrogens and parathormone upon calcium metabolism, Ranney (*Endocrinology* **65**, *594 (1959)*) employed the same Ca^{45} technique. Dividing 15 groups of ten adult male mice into three groups of 50 each, he administered 1.2 mg. of estrone per kg. of body weight to group one, 165 USP units of parathyroid extract per kg. of body weight to group two, and solvent oil to group three for a total of 23 days. Then he gave subcutaneous injections of Ca^{45} lactate and obtained samples of serum and of bone. By calculation, he estimated the rate of bone accretion of calcium, the total exchangeable fraction (pool) of calcium, and the rate of resorption of calcium from bone.

His results confirmed the report of Manunta, Saroff and Turner that estrogens and parathormone had opposite effects upon the rate of accretion of calcium. However, the differences in the exchangeable fractions of calcium among the three groups were negligible. Administration of both hormones simultaneously resulted in a canceling effect. However, if the dose of parathyroid hormone was kept constant and that of estrone increased progressively, the rate of accretion rose, although less rapidly than in animals given graded doses of estrone alone.

In order to study the size of the exchangeable mineral pool, Ranney gave Na^{22} to a group of animals treated similarly. Estrone did not affect the concentration of Na^{22} in serum but did result in greater amounts in bone. This was considered as indicating an expansion of the exchangeable calcium pool.

The author concluded that his results indicated little or no effect of parathyroid extract upon the exchangeable calcium pool. He suggested that the antagonistic action between estrone and parathyroid extract respecting the rate of bone accretion of calcium was caused by (1) an increase in the size of the exchangeable (calcium) pool by estrone, (2) the relative insensitivity of animals to small quantities of estrone but greater sensitivity to larger doses, and (3) the "action of estrogens upon bone (which) may in some way promote the inclusion of calcium citrate complexes into the stable phase of bone crystal."

This study confirms the fact that parathyroid hormone does produce demineralization of bone and that estrogens have an opposite effect. However, the assumption that Na^{22} and Ca^{45} occupy the same exchangeable bone fraction seems presumptuous. Also there would seem to be little reason to invoke theories of variable sensitivity to estrone or of alterations in citrate metabolism unless these functions were measured. While this article adds slight information to the problem of calcium metabolism, the technique of isotopic metabolic study should aid further investigators.

VITAMIN B_{12}, METHIONINE AND FAT

Vitamin B_{12} and methionine are both shown to increase utilization of fat in the diet. The nature of their interrelationships and their roles in the utilization of fat remain obscure.

It has been shown that raising the fat level of corn-soybean meal diet from 3 to 22 per cent will increase the severity of vitamin B_{12} deficiency in chicks (M. R. Spivey *et al.*, *Proc. Soc. Exp. Biol. Med.* **85**, *451 (1954)*), at the same time elevating the vitamin B_{12} requirement (M. R. Spivey Fox *et al.*, *Ibid.* **93**, *501 (1956)*). Fox *et al.* (*J. Nutrition* **62**, *539 (1957)*) have further shown that the high vitamin B_{12} requirement could be eliminated by supplementation of the diet with methionine.

However, the usefulness of the above crude diet for vitamin B_{12} studies was limited because it did not permit the omission or variation in level of most nutrients. To overcome this difficulty, Spivey Fox, L. O. Ortiz and G. M. Briggs (*J. Nutrition* **68,** *371 (1959)*) have studied the vitamin B_{12}-sparing effect of methionine in chicks fed a purified diet containing 0, 4 and 24 per cent hydrogenated vegetable oil.

All diets used in these experiments had the following basal composition in grams per kilogram of diet: soybean protein 300, L-cystine 3, corn oil 40, salts A 60, vitamins 1, and crude glucose 596. The following vitamins were added in milligrams per kilogram of diet: thiamine hydrochloride 8, riboflavin 8, calcium pantothenate 20, choline chloride 1000, nicotinic acid 100, pyridoxine hydrochloride 8, D-biotin 0.3, pteroylglutamic acid 3, vitamin A acetate 6, vitamin D_3 0.02, alpha-tocopherol (free) 25, alpha-tocopherol acetate 25, and 2-methyl-1,4-naphthoquinone 1. When vitamin B_{12} was added, it was at a level of 0.1 mg. per kg. of diet. In the fat-free diets, vitamin D_3 and 2-methyl-1,4-naphthoquinone were added to the diet in alcoholic solutions. Vitamins A, E and K were added to the drinking water of chicks fed fat-free diets. Supplements of DL-methionine, ranging from 0.1 to 1.5 per cent of the diet, were added to diets containing each level of fat.

Chicks that received no vitamin B_{12} and no supplemental methionine lost weight as the fat content of the diet was increased. When vitamin B_{12} was added, growth was equally good with either 0, 4 or 24 per cent fat in the diet. With 4 or 24 per cent fat, the addition of methionine at 0.3 and 1 per cent raised the growth rate of vitamin B_{12}-deficient chicks to equal that obtained in chicks receiving vitamin B_{12}. The 1.5 per cent level of methionine was toxic in both fat-containing diets, irrespective of vitamin B_{12} status. Moreover, with both diets, 1 per cent of methionine plus vitamin B_{12} improved the growth rate over that obtained with the vitamin alone. Thus methionine could not completely replace vitamin B_{12}.

The authors emphasize the effect of supplemental methionine in the absence of fat. Methionine did not replace or spare vitamin B_{12}; in fact the 1 per cent level of methionine had an adverse effect upon feathering, leg bone formation and growth. The wing feathers broke very easily, pigmentation was deficient and feathering on the back, breasts and legs was retarded. Vitamin B_{12}, however, partially protected against these defects. The authors are of the opinion that the toxicity of methionine in the fat-free diets may be related to a possible role of methionine in the utilization of fat.

The effects of dietary fat level, methionine supplements and vitamin B_{12} upon food and energy intake were also assayed. Vitamin B_{12}-deficient chicks, unsupplemented with methionine, utilized the diet with equally poor efficiency at each level of fat intake. Thus chicks receiving 24 per cent fat had a considerably higher caloric intake per unit gain. Chicks receiving vitamin B_{12} or methionine utilized the diet efficiently at each level of fat intake and the food intake was lowered so that the caloric intake remained relatively more constant.

No clear cut effect of the various diets could be seen upon the concentration of total fat, phospholipid and total cholesterol in the sera of the various groups of chicks cited above. The livers appeared normal and had normal concentrations of total lipids and phospholipids. The authors conclude that if derangement in fat utilization is involved in producing a vitamin B_{12} deficiency in chicks fed the high-fat diet, more sensitive parameters of response are required to detect it.

The authors suggest these possible interrelationships between vitamin B_{12}, methionine, fat utilization and protein synthesis. The important relationship between vitamin B_{12} and methionine may involve synthesis or transfer of a methyl group from some source other than choline or betaine or both. Also, if vitamin B_{12} does function in protein syn-

thesis, then the effect of vitamin B_{12} in the high fat diets could be attributed to more efficient, but non-specific, utilization of the available methionine.

Further study is required to determine the role of methionine and vitamin B_{12} in fat metabolism. This may be related to their ascribed role in protein synthesis.

A STUDY OF NAIL GROWTH

Since the normal growth rate of nails of rats and man is delayed by protein deficiency and other factors, measurement of nail growth may provide a useful index.

Man has employed both commonplace and ingenious methods for measuring the effects of dietary substances on the body. Among the less common is the measurement of the rate of growth of fingernails. W. B. Bean (*J. Invest. Dermat.* **20,** *27* (*1953*)) studied the rate of growth of his own nails over a period of ten years. He used a simple method; scoring the nail at the cuticular margin to determine the length of time required for the mark to grow out to the free edge. Factors which affected rate of growth were obscure except for retardation subsequent to mumps.

Others have employed more elaborate methods for studying nail growth. W. E. LeGros Clark and L. H. D. Buxton (*Brit. J. Dermat.* **50,** *221* (*1938*)) scored a mark at the margin of the lunula and measured the rate of its progression periodically. By this means they found that nails grew faster in warm weather than in cool, and grew quite rapidly in nail biters. Retardation of growth was observed in poorly nourished children by M. L. Gilchrist and L. H. D. Buxton (*J. Anat.* **73,** *575* (*1939*)).

The rate of growth of fingernails in man has been estimated by these various authors to be between 0.085 and 0.125 mm. per day, the slower rates occurring in cool weather or under conditions of poor nutrition. M. J. Babcock (*J. Nutrition* **55,** *323* (*1955*)) evaluated a number of methods for measurement of nail growth and selected a technique by which migration of a scratch away from the lunula could be photographed and measured under standard conditions. Unfortunately a few persons do not have sharply defined lunulae.

In a study of nail growth in rats, K. O. Godwin (*J. Nutrition* **69,** *121* (*1959*)) employed this technique and others before he selected one. He made a tattoo near the margin of the cuticle using a needle and Evans blue dye. At the same time, he made a shallow cut in the nail adjacent to the cuticular margin. Readings were made at intervals of two to three days with a filar ocular micrometer, and the average daily rate of nail growth was calculated. By this means he could measure sudden changes such as the effect of thermal or emotional stimuli (fright due to close restraint).

Changes in the diet or the environment were found to have an effect on the rat's nail growth similar to those which had been observed in humans. The mean rate of growth observed during feeding of a stock diet (18 per cent protein) was 0.106 mm. per 24 hours. By contrast, animals fed a protein-free diet had a rate of only 0.045 mm. per 24 hours. The dietary protein was increased by increments in successive groups of animals, employing a number of animal and vegetable foods. A significant correlation was found between the net dietary protein value and rate of nail growth.

Comparisons were made between the effects of adequate and inadequate amounts of vitamin A in the diets. It was found that nail growth was retarded significantly by a lack of vitamin A.

Studies also were made of the effects of exposure to cold (6°C) on nail growth. The

rate was reduced sharply regardless of whether the animals were acclimatized or not. Similarly, 5 mg. of cortisone acetate per day reduced nail growth by about half.

Of great interest is the observation that rats fed a diet containing 10 per cent raw *Phascolus vulgaris* (haricot or snap beans) had a complete cessation of nail growth. This legume has been reported to contain a phytohemagglutinin (D. A. Rigas and E. E. Osgood, *J. Biol. Chem.* **212,** *607 (1955)*). Although not considered toxic in the quantities eaten by man, this substance, which is a mixture of a mucoprotein and a euglobulin, is capable, in dilute solutions, of agglutinating erythrocytes. The nutritive value of *P. vulgaris* and of a number of other legumes has been studied with regard to the inadequacy of their proteins due to lack of methionine. The addition of this amino acid resulted in improved growth rates of rats (W. C. Russell *et al.*, *J. Nutrition* **32,** *313 (1946)*; *Nutrition Reviews* **5,** *144 (1947)*), suggesting that the beans were deficient rather than toxic.

Unfortunately Godwin did not add methionine to the diets containing *P. vulgaris* to determine whether it would restore nail growth. He did mention a toxic factor in *P. vulgaris*, but gave no references.

Godwin's techniques and observations are of sufficient interest to workers in nutrition to warrant more than a casual glance. Most dietary studies employ "gain of body weight" in growing animals, or nitrogen balance studies in adult animals or people, to evaluate adequacy of a diet or toxicity of a drug. However, these methods are slow and reflect changes over a period of several days or weeks. If the work of Godwin can be confirmed, then rates of nail growth may provide a day-to-day index.

Nevertheless, much further work will be needed to establish parameters of nail growth under all conditions, and to determine those nutritional factors which regulate this growth. It may be that people and animals have a convenient built-in gauge by which we can read their nutritional status.

AMINO ACID IMBALANCE, I

The growth depression caused by addition of a mixture of amino acids creating an imbalance depends on the rate of gain supported by the original diet.

Although there is some difference of opinion regarding the use of the term amino acid imbalance, A. E. Harper (*J. Nutrition* **68,** *405 (1959)*) has defined this as any change in the proportions of the amino acids in a diet resulting in "an adverse effect which can be prevented by supplementing the diet with a relatively small amount of the most limiting amino acid or acids." According to Harper, this leaves open the question of whether there are different types of amino acid imbalances, *i.e.*, whether imbalances caused by adding relatively small amounts of one or two amino acids to a diet are identical with those produced by adding a relatively large quantity of a protein or of an amino acid mixture lacking a single amino acid. It excludes, however, those conditions described as antagonisms and toxicities in which adverse effects are caused by the addition of a fairly large excess of a single amino acid and which are not known to be prevented by a relatively small supplement of the amino acid that is most limiting for growth.

As the result of preliminary experiments on this subject, Harper has developed an hypothesis which appears to explain why dietary additions that cause quite severe imbalances in low protein diets are almost without effect when the protein level is sufficiently high to satisfy the amino acid

requirements of the experimental subjects. The hypothesis is based on the fact that the growth response to a given increment of the amino acid that is most limiting in a diet diminishes when the growth rate becomes maximal. It follows that if the requirement for the limiting amino acid is increased by a constant amount when a quantity of an amino acid mixture causing an imbalance is added to a diet, then the growth-retarding effect of this addition should diminish as the dietary level of protein approaches adequacy.

Harper tested this hypothesis experimentally by determining the growth rates of animals ingesting diets containing: (1) a constant level of balanced protein but increasing increments of unbalanced protein, (2) a constant level of an unbalanced protein or amino acid mixture but increasing increments of balanced protein, and (3) either of the above proteins with increments of the amino acid most limiting for growth.

Groups of five male weanling rats of the Sprague-Dawly strain were fed the basal diet for three days and were then given the experimental diets ad libitum for a two-week period. The percentage composition of the basal diet was as follows: casein, 6.0; gelatinized corn starch, 83.6; corn oil, 4.5; mineral mixture, 5.0; choline chloride, 0.15; fat soluble vitamin mixture in corn oil, 0.5; and water soluble vitamin mixture in sucrose, 0.25.

It was found that the growth of rats fed a diet containing 6 per cent of casein supplemented with methionine was stimulated by the addition of threonine. Gelatin, which contains threonine but not tryptophan, stimulated growth when added at a level of only 3 per cent. However, when increments of gelatin greater than 3 per cent were added, the rate of gain fell off until, with the addition of 12 per cent of gelatin, the rate of gain was considerably less than that of the group fed only the basal diet. Addition of tryptophan, the most limiting amino acid, not only prevented this growth retardation but stimulated growth above that obtained with the basal diet.

When a 3.45 per cent mixture of amino acids lacking threonine was added to diets containing casein supplemented with methionine, the greatest growth depression occurred when the diet contained 6 per cent of casein. The depression was less when the casein content was decreased to 4 per cent and very little growth depression was observed when the diet contained 15 per cent of casein.

The effect of 4 per cent of an amino acid mixture lacking threonine on the need for threonine by rats fed a diet containing 6 per cent of casein supplemented with 0.3 per cent of DL-methionine was assayed, with the result that somewhere between 0.025 and 0.05 per cent of L-threonine had to be added to overcome the growth depression.

Harper further points out that "if an amino acid mixture causing an imbalance increases the need of the animal for the limiting amino acid by a constant percentage regardless of the original level of protein in the diet, then, although the magnitude of the growth depression caused by adding a stated amount of an unbalanced amino acid mixture to a diet would depend upon the adequacy of the diet, the growth depression should be prevented by the same level of the limiting amino acid in each case."

This reasoning provided the basis for a final experiment in which three levels of casein (4, 7 and 10 per cent) supplemented with 0.3 per cent of DL-methionine in each case were fed. The growth depression was greatest when the diet contained 7 per cent of casein and was less if the diet contained either 4 or 10 per cent of casein. However, regardless of the level of casein, the growth depression was not prevented by the addition of 0.05 per cent of DL-threonine but was completely prevented by an addition of 0.1 per cent. These results are in accord with the proposed theory.

LACTOSE AND CALCIUM ABSORPTION

It appears that the enhancement of calcium absorption by the presence of lactose in the diet is due to a direct action of lactose either on the gut wall or within the intestinal lumen.

The stimulating effect of dietary lactose on calcium absorption has been known for many years. A publication by F. W. Lengemann *et al.* (*J. Nutrition* **61,** *571* (*1957*)) has re-emphasized the favorable effect of milk on calcium and strontium absorption. The metabolism of lactose per se has been reviewed by R. L. Atkinson *et al.* (*J. Dairy Sci.* **40,** *1114* (*1957*)) and others. However, the mechanism by which lactose acts is still obscure.

One of the most popular theories, based upon the fermentation of the poorly absorbed lactose by intestinal bacteria and the subsequent favorable effect of an increased acidity on calcium absorption, has been challenged by P. Fournier *et al.* (*J. Physiol.* (*Paris*) **47,** *351* (*1955*)) and by R. H. Wasserman *et al.* (*J. Nutrition* **62,** *367* (*1957*)). Fournier and associates have shown that many of the sugars that increase calcium retention are those that are attacked with difficulty by intestinal bacteria. Wasserman's argument is based on the observation that chicks do not respond to lactose-containing milk powder whereas rats do show enhancement of calcium absorption. Both of these species have the potential for forming an intestinal bacterial population capable of fermenting glucose rapidly.

Other theories have been proffered as well. Some suggest that lactose acts by diminishing the endogenous secretion of calcium into the intestine, and others that lactose functions as a structural entity to favor bone cell metabolism.

Lengemann, Wasserman and C. L. Comar (*J. Nutrition* **68,** *443* (*1959*)) have recently pooled their efforts to obtain basic information on the manner in which calcium and strontium pass across the gastrointestinal barrier and how this movement is enhanced by lactose. Calcium absorption from the gastrointestinal tract was determined by measuring the uptake of Ca^{45}, Sr^{85}, or both, by femurs of rats after a single oral dose of a solution containing these radioisotopes.

An initial study was designed to indicate whether the lactose effect was dependent upon the type of calcium salt presented. It was found that stimulation of absorption occurred whether the calcium was present as the chloride, gluconate, lactate or acetate salt. The authors point out that this is in contrast with the data of E. Roberts and A. A. Christman (*J. Biol. Chem.* **145,** *267* (*1942*)), who reported that lactose had no effect on the absorption of the lactate salt in the same species. When the strong chelating agent sodium ethylenediaminetetraacetate was employed, no effect was found on normal Sr^{85} absorption, but at high concentrations it suppressed completely the action of lactose.

It thus seems that lactose acts only on ionized or readily ionizable calcium or strontium. Solubilization does not appear to be a factor, since ethylenediaminetetraacetate chelates are quite soluble. The similarity in rate of absorption in cases where the Sr^{85} is presented alone and where ethylenediaminetetraacetate is added may represent that component of the total absorption mechanism of alkaline earths caused by a diffusion-like mechanism.

To determine a possible effect of ethylenediaminetetraacetate on movement of calcium between the blood and the skeleton, the animals in two groups were given Sr^{85} intraperitoneally rather than orally. There was no effect on bone deposition of Sr^{85} in the group to which lactose had been administered.

When the absorption of Sr^{85} was deter-

mined as a function of time following oral administration, it was observed that lactose exerted its effect quite rapidly. In as short a time as two hours following ingestion, the femurs of the lactose-treated rats contained more Sr^{85} than did those of the control animals. Surprisingly, when the stomachs of the rats were excised to determine the influence of lactose on gastric emptying time, it was found that the presence of lactose resulted in a decreased rate of removal of Sr^{85} from the stomach. At 30 minutes, 80 versus 90 per cent of the activity in the respective groups had left the stomach.

The authors are of the opinion that this slowing of the rate at which the substance reaches the site of absorption cannot alone explain any enhancing effect of lactose on the extent of calcium absorption.

In one experiment, solutions were injected into ligated segments of the gastrointestinal tract. Lactose enhanced absorption most effectively in the ileum (a 4-fold increase) but an appreciable response was seen in the duodenum (a 1.3-fold increase) and the jejunum (a 1.4-fold increase). In the ileum a lactose effect on the femur was apparent at 30 minutes. However, the difference between lactose-injected and control rats was only barely significant at this time interval. The difference at one hour was much more marked, and at two hours the differences were highly significant.

It is well known that calcium is readily absorbed from the upper portions of the small intestine and less efficiently from the ileum. Since the total effect of lactose in increasing calcium absorption is about a factor of 2.0 and the relative enhanced absorption from the segments of upper intestine was about 1.4, in contrast to 4.0 for the ileum, the authors reason that the main anatomical site of lactose action is the ileum. This means that the calcium absorbed by the ileum and acted upon by lactose is that which has escaped absorption in the upper portions.

The authors point out that the ileum has been suggested as the important site for the primary action of vitamin D in the absorption of calcium. Although vitamin D-deficient chicks and rabbits failed to respond to lactose, groups of rachitic rats showed increased absorption in the presence or absence of vitamin D when lactose was given. Apparently there is a species difference, and it would be of interest to investigate further the metabolic differences in the ileums of these species and the precise relationship between vitamin D and lactose.

The authors believe that these results tend to indicate that lactose does not act by providing substrates for acid fermentation by bacteria. In the first place, the time for the appearance of an increase in calcium absorption due to lactose seems too short for a significant amount of sugar fermentation to have occurred. Secondly, the rats had been raised on a lactose-free ration. Thus, it seems unlikely that they would possess an intestinal flora that readily attacks lactose. In addition, when the authors gave a diet containing 1 per cent of sulfadiazine for one week, they nevertheless observed an enhancing effect of lactose.

From these experiments, no definitive statements can be made concerning the exact mechanism by which lactose acts on the calcium transport mechanism. The conditions were such that lactose could not have elicited the observed response through an effect on bone. The ratio between femur Sr^{85} and absorbed Sr^{85} was not altered appreciably in the presence of lactose. Moreover, the manner by which bone-seeking radioisotopes enter the skeleton (*i.e.*, by an ion exchange and adsorption phenomena) discounts any skeletal effect of lactose. Hence the authors conclude that lactose has a direct action either on the gut wall or within the intestinal lumen.

STARVATION AND BODY ELECTROLYTES

Serum and carcass electrolyte concentrations in rats following acute or semi-starvation depend on the extent of weight loss. Severe weight loss results in lowered plasma potassium and an increase in carcass chloride concentrations.

In an effort to determine the metabolic changes associated with hypokalemia and hyponatremia seen in anorexia patients (*Nutrition Reviews* **18,** *71 (1960)*), J. R. Elkinton and E. M. Widdowson (*Metabolism* **8,** *404 (1959)*) subjected groups of adult rats (body weights ranged from 238 to 355 g.) to a restricted intake of a stock diet. These rats lost 4 per cent of their body weight each week. At the end of seven weeks, when the body weight loss amounted to 30 per cent, four of the rats were killed. The serum from these animals showed no changes in chloride, sodium, potassium, calcium or magnesium concentrations when compared with the ad libitum-fed controls.

Another group of four rats that had lost 30 per cent of their body weight were given 400 mg. of sodium chloride each day during the seventh week. At the end of that period, the concentration of electrolytes in their plasma was also normal. Magnesium hydroxide feeding under similar circumstances (120 mg. per day) also had no effect on the level of plasma electrolytes.

When the rats were maintained on the restricted food intake for a total of 13 weeks, by which time they had lost 44 per cent of their body weight, the plasma potassium was reduced from 4.7 to 2.9 mEq per liter in the ad libitum-fed controls. The levels of other plasma electrolytes in these rats were normal.

Analyses of the carcasses of the rats that had been semi-starved for seven and 13 weeks showed a reduction in the potassium concentration. The loss of carcass potassium was greater than that of nitrogen as shown by a progressive reduction in the potassium to nitrogen ratio from 2.43 to 2.27 to 1.96 in the rats semi-starved for zero, seven and 13 weeks respectively. Associated with this reduction in potassium was an increase in carcass chloride concentration, which was 30.8 mEq per kg. of fat-free wet tissue in the control rats but which increased to 37.0 mEq in the rats semi-starved seven weeks and to 40.7 mEq in those semi-starved thirteen weeks. An even greater percentage increase in chloride concentration occurred in the skin. The values for the three groups were 62.0, 77.7 and 90.3 mEq per kg. of fat-free skin respectively. The concentration of sodium tended to follow the chloride levels in the carcass and skin, but the changes were considerably smaller.

There was no significant increase in the concentration of sodium in the muscles of the semi-starved rats, whereas the chloride concentration did increase. This observation suggested to Elkinton and Widdowson the possibility that in the skeletal muscle of starved rats, chloride is sequestered in a manner which prevents the establishment of an equilibrium with the plasma chloride.

The rats that had been semi-starved for six weeks and then given large amounts of sodium chloride on each of seven days, showed no increase in body water when compared with the rats that were semi-starved without the salt loading. Thus in this respect the rat differs very markedly from semi-starved human beings, who retain water when salt is given and may go on to develop visible edema (A. Keys *et al., The Biology of Human Starvation, vol. 2, p. 921. Univ. of Minnesota Press (1951)*).

E. J. Huth and Elkinton (*Am. J. Physiol.* **196,** *299 (1959)*) extended the above studies to seven adult rats that had been deprived of all food for three to six days (although water consumption was permitted). As a re-

sult of the starvation, the rats lost approximately 32 per cent of their original weight. The six control rats killed at the start of the experiment had 10.2 per cent fat in their carcasses, whereas the starved rats had 1.3 per cent. In spite of the marked loss of body weight and fat, the composition of the lean body mass in both the control and starved rats was essentially the same with respect to nitrogen (3.7 and 3.8 per cent respectively) and water (72.1 and 71.2 per cent respectively).

Serum collected from the starved rats just prior to the end of the experiment had the same concentration of sodium, chloride and carbon dioxide as the control animals. The potassium level was slightly lower in the serum of the starved rats than in the controls (3.9 versus 4.9 mEq per liter respectively).

Carcass analyses showed that the concentration of chloride was significantly higher in the starved rats than in the controls (42.6 mEq versus 36.6 mEq per kg. of fat-free wet tissue), while the concentration of sodium was only slightly increased in the starved rats (59.7 versus 54.7 mEq respectively). Potassium concentration, on the other hand, was slightly reduced in the bodies of the rats that had been starved (75.1 versus 81.6 mEq). These changes are similar to those seen by Elkinton and Widdowson (*loc. cit.*) in their semi-starved rats.

Samples of muscle removed from the thighs of the starved rats showed a significant increase in the chloride concentration (16.0 for the starved rats versus 13.8 mEq per kg. of fat-free wet muscle for the controls). Associated therewith was an increase in the extracellular water from 123 g. per kg. of fat-free muscle in the controls to 139 g. in the starved rats. Calculations for these extracellular fluid volumes were based on the assumption that the extracellular fluid is equivalent to the chloride space. The concentrations of sodium, potassium and nitrogen in the thigh muscle showed no alteration during starvation.

The above data suggest that in acute starvation resulting from the complete absence of food, the extracellular fluid appears to increase at the expense of the intracellular. Huth and Elkinton explain the difference between the electrolyte changes in the thigh muscle and those in the fat-free carcass as caused by the higher proportion of non-metabolizable solids in the carcass. This resulted in a decrease in the water content of the fat-free carcass, while that of the fat-free skeletal muscle increased.

An extensive study of acute and semi-starvation in rats was made by E. M. Widdowson and R. A. McCance (*Brit. J. Nutrition* **10,** *363 (1956)*). The authors were primarily interested in determining whether the two conditions produced similar biochemical changes. The frequent occurrence of edema in chronic or semi-starvation and its absence in individuals undergoing acute starvation is the most prominent difference between these two conditions observed in human beings.

These British investigators fed a nutritionally adequate diet composed of natural foods to six adult male (315 g. body weight) and six female (220 g. body weight) rats. The food intake of these animals was restricted so that their body weights at the end of six weeks would be the same as that of animals fed the same diet on an ad libitum basis for five weeks and then subjected to six days without food. A control group fed the same diet on an ad libitum basis for five and one half weeks provided the base line data. The latter group showed only a slight increase in body weight, whereas the other two groups lost 15 per cent of their body weights.

Carcass analyses showed that the animals whose food intake had been restricted for the six-week period were similar to those that had been without food for the last six days of the experiment. This was true for protein, sodium, potassium, calcium, magnesium, chloride and phosphorus. There were slight differences for some of these elements

between the semi- and acutely-starved rats, but these differences did not appear significant and were not consistent for the sexes. In most cases, the reduction in the concentration of these electrolytes was the same as the reduction in body weight.

A possible explanation for the failure of the rats starved by Widdowson and McCance to show significant changes in potassium may be the relatively small percentage of body weight lost by their rats. Although the initial weights of the rats used by both groups of investigators were essentially the same, the weight loss of the rats used in the British study was only 15 per cent, while in the American study it was 32 per cent. Moreover, there were marked differences in the percentages of body fat lost. The rats starved by Widdowson and McCance lost, at the most, 40 per cent of their fat stores, while the rats starved by Huth and Elkinton lost 90 per cent of their fat stores.

It is possible that the disturbances in potassium metabolism occur only when the body weight and body fat losses become of considerable magnitude. This would be consistent with the results of studies with anorexia nervosa patients, in whom changes in serum electrolytes were observed only when the body weight loss exceeded 25 per cent (*Nutrition Reviews* **18,** *71 (1960)*). Nevertheless, while some of the results with rats parallel the alterations in potassium metabolism seen in anorexia nervosa patients, it remains for future work to elucidate the mechanisms that bring about these conditions.

NUTRITIVE VALUE OF FRYING OILS

Although at least three types of toxic materials may be produced by overheating or oxidizing oils, hydrogenated frying oils used commercially may retain their nutritive value.

Interest in the possible deleterious effects of overheated fats has continued unabated for over 20 years (see, for example, *Nutrition Reviews* **2,** *18 (1944)*; G. O. Burr and R. H. Barnes, *Physiol. Rev.* **23,** *256 (1943)*). However, enthusiasm has not generally been matched by accomplishment and, with few exceptions, little real progress can be claimed.

There is still some disagreement, for example, concerning the types of compounds responsible for the toxic effects of the overheated fats, with some research workers claiming peroxides as the sole toxic agents and others placing the entire blame on polymeric products (*Nutrition Reviews* **14,** *28, 122 (1956)*; **15,** *346 (1957)*). From a perusal of the literature, however, it seems obvious that both types of products are toxic, the peroxides being produced largely under conditions of moderate temperature in the presence of air, while the polymeric type products (probably cyclic monomers) are produced under high-temperature anaerobic conditions.

Nevertheless, two questions of primary importance have not been answered at all. First, what is the damaging mechanism in both types of toxic agents and, second, what are the amounts of such agents actually formed in fats used in standard cooking processes?

In a recent paper, E. G. Perkins and F. A. Kummerow (*J. Nutrition* **68,** *101 (1959)*) reported an investigation of the nutritive properties of corn oil stirred with air at 200°C for 48 hours. This thermally oxidized oil was converted to the fatty acids, which were separated by urea fractionation into an adduct-forming portion (64 per cent, probably straight-chain acids) and a non-urea-adduct-forming portion (36 per cent, probably polyunsaturated and branched or cyclic acids or polymeric products). The latter fraction was subjected to molecular distillation at 1 to 3

microns pressure and 150°C, thus separating monomeric from polymeric fractions.

All fractions were fed to weanling rats as 12 per cent of a diet consisting of 50 per cent glucose, 31 per cent casein, 2 per cent additional fat (fresh cottonseed oil), 5 per cent salt mixture and adequate supplements of water- and fat-soluble vitamins.

The rats on the non-distillable, non-urea-adduct-forming fraction lost weight and died within seven days. Those on fresh corn oil fatty acids, the urea-adduct-forming fraction or the molecular distillate of the non-urea-adduct-forming fraction all gained weight normally and appeared in good health. Growth was also severely depressed by the total thermally oxidized fatty acids or the total non-urea-adduct-forming fraction. Dilution of the latter with an equal weight of fresh corn oil fatty acids alleviated, but did not prevent, the growth-inhibiting action.

The authors suggest that the toxic fraction, which represents 30.6 per cent of the original oil, is a relatively small proportion and that most of the oil is undamaged by the treatment. It is possible that the toxic material is actually present in considerably smaller amounts since it has been reported previously that the actual polymeric fractions formed during anaerobic heating to 275°C are not very toxic (*Nutrition Reviews* **15,** *346 (1957)*). In this case, therefore, only part of the polymers may be responsible for the effects.

It might also be proposed that not two but three distinct toxic products are produced by different treatment of the oil. First, at high temperatures in the absence of oxygen, the cyclic monomer is the major toxic substance. On the other hand, hydroperoxides are the major toxic substance after treatment at moderate temperatures for short duration in the presence of oxygen. Moreover, it has been shown in the present work that at high temperatures in the presence of oxygen, toxic oxygen-containing polymers are produced.

The authors do not state that the conditions employed in these studies duplicate those of actual frying processes, but they point out that caution should be exercised in the dietary use of unsaturated oils which have been subjected to such treatment.

In a study of fats actually used in frying, K. W. Keane, G. A. Jacobson and C. H. Krieger (*J. Nutrition* **68,** *57 (1959)*) determined the caloric value of a hydrogenated cottonseed oil which had been used for periods of 14 to 24 days in a commercial frying operation. The used or fresh oil was incorporated as 23 per cent of a basal diet composed of 20 per cent casein, 73.6 per cent sucrose, 2 per cent cottonseed oil, 4 per cent mineral mixture and adequate supplements of water- and fat-soluble vitamins.

It was found, somewhat surprisingly, that the caloric values of the oil samples, based on the caloric restriction technique described by E. E. Rice, W. D. Warner, P. E. Mode and C. E. Poling (*J. Nutrition* **61,** *25 (1957)*), were directly related to the length of use of the oil in the frying process. Searching for some chemical changes in the oil samples which might account for the changes in their nutritive properties, the authors found that the total non-conjugated double bonds increased in direct proportion to the caloric value. The same relationship was also shown to hold for different stages of hydrogenation of cottonseed oil.

It was evident from these experiments, therefore, as shown previously, that caloric availability of fats is related to their unsaturation (see, for example, *Present Knowledge in Nutrition, The Nutrition Foundation, Inc., 1956*). In this case, however, the mechanism of double bond production was of interest, since it is usually found that heated or aerated fats show decreases in unsaturation because of peroxidation or polymerization.

In an analysis of the alterations in double bonds during 18 days' use of the fat, it was found that there was an increase in dienoic and possibly conjugated dienoic acids, but little change in any of the other polyunsaturated acids. The reasons for these in-

creases, however, are uncertain since the authors intimate that there was probably a considerable contribution from the fat of the chicken parts being fried but do not include quantitative data. Moreover, fat was being replaced at an unspecified rate and no assessment was made of the contribution of this process. No logical explanation, therefore, can be given for the changes in unsaturation, particularly since the temperature and other conditions were not stated.

In a further evaluation of the used hydrogenated cottonseed oil, Keane and co-workers carried out experiments in which weanling rats were fed the basal diet with 18 per cent of variously treated samples of hydrogenated cottonseed oil with or without an additional 2 or 4 per cent cottonseed oil as a source of essential fatty acids. It was found, in confirmation of the previous results, that growth rate for five weeks was greatest for the used hydrogenated cottonseed oil. Moreover, the additional cottonseed oil gave greater growth rate only if the hydrogenated oil had been "laboratory heated and oxidized" and gave a subnormal growth response. Even in this case, no symptoms of toxicity such as diarrhea or rough hair coat appeared.

When the same results were obtained with corn oil (which, however, was an old sample and gave poor growth response) the authors felt the need of explaining the differences between these findings and those of other workers, who usually obtain low caloric values and toxic symptoms with heated or oxidized oils. In a comparison of their diet with that used by O. C. Johnson, T. Sakuragi and Kummerow (*J. Am. Oil Chem. Soc.* **33,** *433 (1956)*), Keane and co-workers pointed out that their own diet contained additional cystine, glycine, inositol, niacin, para-aminobenzoic acid and biotin while these substances were lacking in the diet of Johnson and co-workers, thus possibly accounting for the toxic effect of the oxidized oil.

However, there may be other explanations for the discrepancies not readily apparent. It is to be expected that a hydrogenated oil will not suffer decomposition on heating as readily as a highly unsaturated oil. Moreover, if a fat high in linoleic acid (such as chicken fat) is being continuously added, the results will not be strictly comparable to the simple case.

The authors did not indicate what was meant by a "laboratory heated and oxidized" oil nor did they describe the properties of the product of this treatment, which might have facilitated comparison. It is certainly true that the composition of the remainder of the diet has a great deal to do with the effect of the fat, but the literature is almost universally agreed that overheated and oxidized unsaturated oils are detrimental to the well-being of animals.

The value of these studies lies in the information that an actual commercial frying operation has apparently not destroyed the nutritional value of the fat used in cooking but may even have increased it. As the authors state, there is a need for further research on this problem.

VITAMIN B_{12} DEFICIENCY

A high percentage of the offspring of vitamin B_{12}-deficient rats was hydrocephalic and showed other evidences of organ retardation at birth.

L. R. Richardson and A. G. Hogan's experiments on inadequate maternal nutrition (*J. Nutrition* **32,** *459 (1946)*) led subsequent investigators to suspect that a deficiency of either folic acid, vitamin B_{12}, or both was responsible for the resulting hydrocephalus in infant rats. Although B. L. O'Dell, J. R. Whitley and Hogan (*Proc. Soc. Exp. Biol.*

Med. **69,** *272 (1948)*) demonstrated that a deficiency of folic acid was the chief cause of the congenital abnormality, it was later found (O'Dell, Whitley and Hogan, *Ibid.*, **76,** *349 (1951)*) that a high proportion of offspring are afflicted with abnormalities if the mothers are severely depleted of vitamin B_{12}.

P M. Newberne and O'Dell (*J. Nutrition* **68,** *343 (1959)*) have described in considerable detail the pathology of the central nervous system of vitamin B_{12}-deficient rat embryos and have included observations on the peripheral nerves, lungs, kidneys and adrenal glands from vitamin B_{12}-deficient and control embryos.

To deplete the body stores of vitamin B_{12}, female rats were fed, from weaning, a diet of the following composition: soybean oil meal (70 per cent), glucose (22 per cent), lard (4 per cent), salts (4 per cent). Vitamins given (in milligrams per 100 g. of diet) were: thiamine hydrochloride, 1.6; riboflavin, 1.6; pyridoxine hydrochloride, 1.6; calcium pantothenate, 4.0; choline chloride, 100; biotin, 0.02; folacin, 0.5; alpha-tocopherol, 3.0; 2-methyl-1,4-naphthoquinone, 1.0; vitamin A, 2000 I.U., and vitamin D, 280 I.U. The control animals were fed the basal diet supplemented with 30 mg. of vitamin B_{12} per kilogram of diet. The criteria used to determine the deficiency state were a high incidence of hydrocephalus and a mortality of 80 to 90 per cent during the first week.

In the hydrocephalic newborn rat the parenchyma of the brain was spongy and areolar with many distended spaces. The neurons were shrunken and nuclei showed vacuolation. Cytoplasmic changes ranged from slight loss of Nissl substance to gross chromatolysis. The glia showed some proliferation of oligodendroglia which, together with the astrocytes, appeared swollen and vacuolated with degenerative changes in some cases. Using special stains, the authors were able to demonstrate a complete lack of glycogen in the brain of experimental animals as compared to considerable amounts in the choroid plexi and connective tissues of controls.

Adrenal glands were consistently smaller in the experimental animals than in controls of the same body weight. The capsule was thin while the zona arcuata was more pronounced than normal, apparently because of super-staining pyknotic nuclei in the deficient animal. Nuclei of the fascicular zone were pale and the cytoplasm of these cells was greatly distended with lipid, but the adrenal medulla showed no significant deviation from the normal.

The lungs of the experimental offspring typically showed delayed development with poor differentiation into airspaces and considerable distance between the capillaries and their alveolar walls. However, the kidneys exhibited more retardation of growth than any other organ. The embryonic-like cortex consisted mainly of poorly differentiated glomeruli and tubules, the proximal ones being more mature but grossly dilated. C. C. Jones *et al.* (*Proc. Soc. Exp. Biol. Med.* **90,** *135 (1955)*) has made similar observations.

Although myelination of the central nervous system did not seem to be affected by the state of deficiency, the spinal cord consistently showed areas without myelin and the more peripheral nerves revealed either a complete absence or very little myelination of the fibers. The Schwann cells of the myelin sheath appeared to be more numerous in the B_{12}-deficient animals, but fibrosis was not observed.

Histological study of embryonic brains of rats of different ages revealed no discernible changes in the brains of 14-day embryos as a result of maternal deficiency of vitamin B_{12}. By the sixteenth day of gestation approximately 25 per cent of the embryos showed some degree of hydrocephalus with marked alterations in the aqueduct. The most important finding was a larger number of mitotic figures in the aqueductal ependyma of the deficient brains on the sixteenth day of gestation. This is evidence that the abnormal histological pattern developed in

the deficient brain sometime between the fourteenth and sixteenth day of gestation.

The actual hydrocephalus resulting from a vitamin B_{12} deficiency appears to be caused primarily by constriction or closure of the cerebral aqueduct. The authors suggest that the greater number of mitotic figures in the aqueductal area in the 16-day embryonic brain indicates that mitosis is slowed in this particular area (the cells are perhaps held in states of arrested mitosis), and that continued growth of near normal brain tissue constricts the aqueduct and thus precipitates hydrocephalus.

THE PROTEIN SYNTHESIS MECHANISM IN THE PROTEIN-DEPLETED DOG

Methionine labeled with S^{35} was injected into normal and protein-depleted dogs. With depletion increasing to a loss of 25 per cent of body nitrogen, plasma proteins showed increasing radioactivity.

Nitrogen equilibrium can be maintained in an adult dog with 250 mg. of nitrogen as casein and 80 calories per kg. body weight per day (J. B. Allison, J. A. Anderson and R. D. Seeley, *Ann. N. Y. Acad. Sci.* **47,** *245 (1946)*). Isocaloric replacement of protein with carbohydrate does not eliminate loss of nitrogen in the urine but the rate of loss becomes less. The urinary nitrogen falls to a minimum of about 60 mg. per kg. by the sixth week of depletion. As the catabolism of body protein continues, the animal becomes weak, anorexic and edematous. Unless refeeding is done early enough the dog may die in spite of any measures.

Depletion may cause irreversible damage to the protein synthetic mechanism (Allison, *Symposia on Nutrition, vol. 2, p. 123. C. C. Thomas, 1950*). Similar irreversible damage to protein metabolism may sometimes be seen in man.

Kwashiorkor is a clinical syndrome found in human infants in many tropical countries. After finishing breast feeding, infants are often transferred to diets adequate in calories but poor in protein. In some instances the resulting protein depletion may continue too long to be reversible by protein and electrolyte repletion.

In relation to this problem, J. S. Garrow (*J. Clin. Invest.* **38,** *1241 (1959)*) has reported experiments attempting to correlate the degree of protein-depletion with changes in protein metabolism detectable with isotopic tracers.

Eight clinically normal adult dogs were maintained in nitrogen equilibrium on a synthetic diet containing 250 mg. nitrogen (as casein) and 80 calories per kg. body weight per day. Each was injected intravenously with 4×10^6 counts per minute in 0.5 mg. S^{35} methionine and 4 mg. of T-1824 dye per kg. body weight. Four received labeled DL-methionine and four received labeled L-methionine. Two animals in each group of four were started on a protein-free diet on the day of injection and two were started one week later.

During depletion the dogs were reinjected with T-1824 and S^{35} methionine after intervals of three to ten weeks. Four of the dogs were injected a third time after a similar interval. When bleeding was used to accelerate depletion, enough time was allowed between bleeding and injection to permit equilibration of the protein pools.

The cumulative loss of nitrogen was measured in daily urine and weekly feces collections and in blood collected. The dye concentration in plasma was measured in samples taken one-quarter hour and one hour after injection. Plasma volume was measured by extrapolation to zero time.

Radioactivity measurements were made on precipitated plasma proteins and the supernatant. After precipitating the proteins

with trichloroacetic acid they were redissolved in sodium hydroxide and sampled for radioactivity determinations. When reinjections were done, the preinjection residual activity was subtracted from the subsequent values determined after injection. Radioactivity from S^{35} was expressed as specific activity per mg. nitrogen. If one assumes that the sulfur to nitrogen ratio in protein is constant, the figure is proportional to the true specific activity.

The depletion program lasting from 50 to 60 days resulted in a loss of 30 per cent of body nitrogen. Body weight lost ranged from 13 to 18 per cent. The fact that this value is so much less than the per cent loss of nitrogen indicates that some nitrogen-poor component is being retained, *e.g.*, edema fluid. From 20 to 25 per cent of all the nitrogen lost was in the feces, and the nitrogen lost daily averaged about 0.5 per cent of the total.

During depletion of four of the dogs, plasma volume and protein concentration was measured. The circulating plasma protein was rapidly reduced in the later stages of depletion, exceeding the rate of loss of total body nitrogen.

Radioactivity from protein-free plasma fell from 4500 to 350 counts per minute per ml. during an 8-hour period after injection. There was no significant difference between groups either before or after depletion or with L- or DL-methionine.

The amount of incorporation of either L- or DL-methionine into plasma protein was the same. Curves of activity at one, two, four, six and eight hours following injection were similar. However, the height of the curve was much greater in the depleted dogs. Peak activity increased with increasing depletion. The possibility that this increase in peak activity finally reaches a maximum and then declines was suggested in a comparison of 20-per-cent-depleted animals with 32-per-cent-depleted animals. Plasma protein decay curves were not different in normal and depleted dogs. Excretion of labeled sulfur was essentially similar in both groups, although the depleted dogs appeared to excrete the label a little more rapidly. The specific activity of the urine reflected that of plasma protein.

Two factors may account for the difference in specific activity of plasma protein with increasing depletion. One is the reduction in the size of the plasma protein pool, the other is an increased proportion of labeled molecules entering this pool. Blood volume determinations revealed that when 30 per cent of body nitrogen had been lost the circulating plasma protein was half the normal amount, although this was not enough to explain the increased specific activity of the plasma proteins. When 25 per cent of body nitrogen had been lost the greatest amount of incorporation of the radioactive dose occurred.

C. L. Yuile, F. V. Lucas, R. D. Neubecker and G. H. Whipple (*Fed. Proc.* **14**, *424 (1955)*) reported that dogs depleted by plasmapheresis as well as low-protein feeding showed a greater reduction in the extravascular extracellular protein pool than the circulating plasma protein pool. Garrow, however, does not believe that reduction of the extravascular pool could account for increased activity of intravascular protein at six to eight hours after injection, since at least two days are required to equilibrate the two pools.

As a result of analytical techniques, the absolute quantity of radioactivity in intravascular protein may be expressed as per cent of original dose. In normal dogs the blood proteins account for 4.5 per cent of the injected dose. For the second and third injections the averages are 7.2 and 6.5 per cent. Thus with depletion the blood proteins represent increased amounts of the dose.

Study of the excretion of radioactivity permitted a partial evaluation of the fate of the injected label. As depletion progressed, nitrogen excretion diminished and isotope excretion increased slightly. Three assumptions were made in discussing the implica-

tions in this experiment: (a) free and protein-bound methionine reach equilibrium by the seventh day after injection; (b) all labeled sulfur not excreted has been incorporated in protein by the seventh day; and (c) other amino acids behave in a similar manner.

The results indicate that urine and plasma protein specific activities are similar and increase with depletion. Total body radioactivity in counts per minute per mg. of nitrogen resembles the activities of normal urine and plasma protein, but depleted dogs show two to three times the average body radioactivity in their urine and plasma protein.

The fact that even severely depleted dogs excrete as much as 80 mg. nitrogen per kg. body weight daily, which in a 5 kg. dog would be about 2.5 g. nitrogen per day, makes it seem unlikely that the plasma protein would be the main source since in the dog it contains only about 10 g. of protein. This high activity of urinary nitrogen must then come from some fairly large high activity pool.

During the early stages of protein depletion readily available protein from liver and some from plasma is expended first. Later the proteins of slower turnover are used to furnish priority tissues with essential amino acids. Just as destruction of protein shifts during depletion so does the synthesis of protein shift to tissues important to the survival of the organism.

As depletion progresses the loss of important cell enzymes may result in irreversible damage to synthetic mechanisms. Thus the redistribution of anabolic potential may be a more important facet of the concept of protein depletion than the state of the protein stores.

INFLUENCE OF BODY COMPOSITION ON SURVIVAL UNDER STRESS

Piglets fed diets with varying amounts of fat and protein were later subjected to fatal stress (lack of food, lack of water, or both). The results may prove invaluable in planning for human survival.

The problem of survival is of great concern to our armed forces, as well as to civilians in times of disaster or war. Aside from physical injury or exposure to extremes of heat or cold, the greatest hazard is deprivation of water, since man can live for a considerable period if deprived of food alone.

L. S. Bauer and L. J. Filer, Jr. (*J. Nutrition* **69,** *128 (1959)*) have studied the influence of diet upon the survival of baby pigs undergoing three types of deprivation. They selected three-day-old piglets and fed half of them a diet which was high in fat and low in protein while the other half received a diet high in protein but low in fat. All animals were allowed to eat and drink at will. At the end of eight weeks, some of the animals from each group were killed in order to determine their body composition. Those fed the high-fat, low-protein diet had more body fat while those fed the high-protein, low-fat diet had more body water and body protein.

The remaining animals were subjected to one of three kinds of stress: complete deprivation of food and water (stress A), deprivation of water alone with dry food available (stress B), and deprivation of food alone with water available (stress C). Under stress A (complete deprivation of food and water) the pigs fed a high-protein diet lived 28 days, compared with 21 days for the group fed a high-fat diet. However, there was no difference in survival of the two groups when stress B (deprivation of water) was applied; the animals lived 15 to 16 days. By contrast, stress C (deprivation of food) resulted in a marked difference. Under these conditions, the group fed a high-fat diet lived 89 days while those fed a high-protein diet lived only 36 days.

Analysis of the carcasses of these animals

disclosed several significant findings. When deprived of food but with water available (stress C) the pigs used almost all of their body stores of fat and much of their stores of protein. However, as the fatty stores were depleted, the content of water in the body increased. Under all conditions of stress, the weight of the heart tended to increase while the weight of the spleen decreased. The liver became smaller in pigs which were starved.

Studies of electrolytes (sodium, potassium and chloride) in the carcasses of the pigs revealed that complete deprivation of food and water (stress A) caused the sodium and chloride content to rise and the potassium content to fall. Starvation with water available (stress C) resulted in the same changes in electrolyte content but to a greater degree, especially in the animals fed a high-fat diet. It must be remembered, however, that these pigs lived much longer than those under stress A.

Urine, which was collected from a few members of each group, changed in specific gravity, total volume, and in osometric readings as follows. Those pigs deprived of water and food or of water alone voided highly concentrated urine. Those deprived of food alone excreted dilute urine, but the pigs fed the high-fat, low-protein diet excreted much less urine than did those fed the high-protein, low-fat diet.

It was surprising that pigs deprived of water would continue to eat food for ten days, even though this food aggravated their dehydration.

The following conclusions were drawn from these studies. Under conditions of both starvation and thirst, animals with larger stores of body water (high-protein diet) survived longer. However, under conditions of starvation alone, the reverse was true. This may be explained by the fact that fatty stores can provide energy for longer periods of time than can protein stores, but the latter are accompanied by a larger reserve of water. Even though catabolism of fat results in production of some water, the quantity so produced is insignificant.

It has been believed that, under conditions of starvation, the body selectively burns fat first and catabolizes protein only as a last resort. However, the evidence presented herewith contradicts this belief, provided adequate supplies of water are available.

The authors estimated the rates of catabolism of fat and of protein for animals under each type of stress. They concluded that the daily utilization of fat was reasonably constant under all three forms of stress. However, a different situation resulted respecting protein catabolism; the average loss of protein in animals having the greatest stores of protein was two to three times greater than in those having a lesser amount under all three conditions of stress. Furthermore, protein catabolism continued as a constant rate throughout the entire period of stress, as judged by concentrations of urea in the blood. They surmised that the body, once accustomed to a high-protein intake is unable to "gear down" its metabolic processes in time to permit survival.

This study suggests that our customary high-protein diets may not be ideal to prepare us for survival under conditions of sudden deprivation. It also lends credence to the tales of shipwrecked sailors who described early death for the robust members of a crew and longer survival for their less muscular comrades (assuming water was available).

This study might have been improved had the individual groups of animals been larger and of equal size. Also, since young animals generally have a lesser capacity to conserve water and electrolytes than do adults, it might have been better (though more costly) to have used full-grown pigs. However, these are minor objections to a convincing study.

NOTES

Letter to the Editor

Dear Sir:

I feel that exception must be taken to the statement at the end of the first paragraph of the review "Fibrinolysis and Lipid Metabolism" (*Nutrition Reviews* **17,** *263 (1959)*), as it is not a statement of fact.

In the reference cited by you, Fullarton does not state that hyperlipemia may retard fibrinolysis nor do I state that hyperlipemia may accelerate blood clotting. Indeed, I was at some pains to express the point of view that it was difficult to see how acceleration of the clotting time of blood in a test tube can have a bearing on intravascular clotting.

Neither in the case of Fullarton nor in my case is your reference to our respective original work but, in the one case, to reported remarks at a conference and, in the other, to correspondence. This failure to quote original work I regard as a serious omission on your part, in view of the stated aim of *Nutrition Reviews* to make "available an unbiased, authoritative review."

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South African Institute for Medical Research
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Essential Fatty Acids, Serum Cholesterol and Coprophagy

It has been known for a long time that laboratory animals can obtain several vitamins by coprophagy (*Nutrition Reviews* **7,** *283 (1949)*). The source of such vitamins appears to be largely the intestinal flora, which can, under certain conditions, eliminate the need for an outside source of vitamins (*Ibid.* **16,** *126 (1958)*). R. H. Barnes *et al.* (*J. Nutrition* **63,** *489 (1957)*) have developed a method for prevention of coprophagy in the rat and have applied it to a study of the effect of this practice on the requirements of various vitamins (Barnes, E. Kwong and G. Fiala, *Ibid.* **67,** *599 (1959)*).

Despite this clear-cut evidence that a variety of essential nutrients is obtained or at least augmented by coprophagy in several animal species, no attempt had been made to see if this applied to essential fatty acids. Thus in an extension of their previous work, Barnes, S. Tuthill, Kwong and Fiala (*J. Nutrition* **68,** *121 (1959)*) raised male weanling rats on a fat-free diet with or without supplementary corn oil and with or without access to their feces. Rats on both diets grew more slowly when coprophagy was prevented, and this was especially pronounced in the fat-deficient groups, in which the average difference in weight was about 50 g. Moreover, without coprophagy, fat-deficient rats developed symptoms more rapidly and extensively.

Since serum cholesterol values were found to be low in all animals on the low-fat diet, a further study was made of the effect of different dietary fats on the serum cholesterol of fat-deficient rats. Groups of weanling or young adult rats were given fat-free diets alone or with supplements of 1 per cent corn oil, 15 per cent hydrogenated coconut oil, or both. The weanling rats without supplementary corn oil grew poorly, but, as was expected, the young adults were not affected by the deficiency. In both groups, the greatest growth rates were observed in rats given both corn oil and hydrogenated coconut oil. Serum cholesterol values were lowest for the fat-deficient rats and highest in rats fed the combined corn oil and hydrogenated coconut oil supplements. Especially in the case of the weanling rats, the combined oil supplements produced higher serum cholesterol values than did hydrogenated coconut oil alone.

Although these experiments did not demonstrate how the fecal unsaturated fatty acids were derived, it is evident that they were not absorbed from the cecum or large

intestine and only had an effect if re-ingested with the feces. For rapid production of essential fatty acid deficiency, this factor will have to be taken into consideration.

The effect of the unsaturated fat in potentiating the serum cholesterol elevating effect of the saturated fat is somewhat obscure and should be investigated further. The many complex relationships between serum and tissue cholesterol concentrations and dietary fat are far from solution, and at present each new finding seems only to raise further questions.

New Nutrition Journal to be Published in Germany

Zeitschrift fur Ernahrungswissenschaft-Journal of Nutritional Sciences-Journal des Sciences de la Nutrition will shortly appear, with provision for publication of manuscripts in German, English or French. This journal will provide for a wide coverage in subject matter related to foods and nutrition, including clinical nutrition and dietetics, human, animal and plant nutrition, physiology and biochemistry, nutritional chemistry and food technology, toxicology and hygiene, agriculture and veterinary medicine, preserving, storing and transport techniques, and food statistics.

Editor-in-Chief: Professor Konrad Lang, Institut fur Physiologische Chemie, Mainz, Germany.

Co-editors: E. Abramson, K. Bernhard, J. Bruggemann, H. Dam, W. Droese, A. Hock, J. Kuprianoff, W. Lenkeit, H. Malmros, R. Nicolaysen, L. Schmid, A. I. Virtanen.

Diet and Celiac Syndrome

A well documented paper on the practical dietary management of patients with celiac syndrome by E. B. Mike (*Am. J. Clin. Nutrition* **7**, *463 (1959)*) will be of interest to clinicians and dietitians dealing with such patients. The paper provides a tabulation of basic principles of diet for idiopathic celiac disease and for cystic fibrosis of the pancreas. It further includes a guide for the selection of foods for the diets of patients of various ages with these syndromes, based on experience at Babies Hospital, Columbia-Presbyterian Medical Center, New York City.

The paper is quite detailed, providing meal plans, dietary patterns and an outline of a low-gluten diet. It will serve not only as a handy practical guide but also a ready reference to some 40 papers which give more detailed information on the subject.

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The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

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